

Interaction of microbiology and pathology in women undergoing investigations for infertility

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Background: Cases of endometriosis with no tubal damage are associated with infertility, suggesting an immunological rather than mechanical barrier to reproduction. Laparoscopy and fallopscopy results of clinically asymptomatic women undergoing investigation of infertility were correlated with the outcomes of microbiological screening for *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, *Mycoplasma hominis*, ureaplasma species, *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Chlamydia pneumoniae*.

Methods: A total of 44 women presenting to a hospital IVF service for laparoscopic or laparoscopic/fallopscopic investigation of infertility provided endocervical swabs, fallopian tube washings, and peripheral whole blood for analysis.

Results: Of these 44 women, 15.9% (7) showed evidence of *C. trachomatis* infection as detected by either PCR or EIA serology. Of these 7 women, 5 (71%) had no or mild endometriosis and 2 (29%) had moderate or severe endometriosis. Of the remaining 37 women who showed no evidence of chlamydial infection, 15 (40.5%) had no or mild endometriosis.

Conclusion: Women with infertility, but without severe endometriosis at laparoscopy, showed a trend towards tubal damage and a higher rate of previous *C. trachomatis* infection. Although not statistically significant, this trend would suggest that, where moderate to severe tubal damage is found to be the primary cause of infertility, *C. trachomatis* infection could be a likely cause for such tubal damage.

Key words: *CHLAMYDIA TRACHOMATIS*; ENDOMETRIOSIS; FALLOPOSCOPY; TUBAL INFERTILITY; UREAPLASMAS

INTRODUCTION

Intraluminal pathology of the fallopian tube is correlated with two conditions—pelvic inflammatory disease (PID) and endometriosis. The presence of such tubal disease is a strong

determinant of infertility¹; 20 to 25% of infertile women have been shown to have endometriosis compared with 2 to 5% of fertile women², and up to a third of women with endometriosis show evidence of salpingitis³. Whereas severe cases of endometriosis accompanied by extensive pelvic

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adhesions can lead to tubal infertility, mild or moderate cases with no tubal damage are also associated with infertility, suggesting an immunological rather than a mechanical barrier to reproduction^{4,2}.

PID also causes tubal infertility. The severity of tubal damage occurring during PID determines the probability of infertility: 3% of women with mild tubal damage observed at laparoscopy, 13% with moderate damage, and 29% with severe damage, were identified as having tubal infertility⁵. Despite the high causal relationship between bacterial infection and PID, organisms are rarely isolated in PID cases, and even less frequently in cases of tubal infertility⁶.

Most women with tubal infertility do not have a history of recognised acute salpingitis. However, a number of previous studies investigating acute infections, pelvic inflammatory disease and salpingitis have demonstrated that *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the most common causes of such infection^{7–10}; and others have demonstrated an abnormally high prevalence of antichlamydial (*C. trachomatis*) antibodies in women with tubal infertility and ectopic pregnancy^{11–15}.

In this study we investigated the severity of intraluminal disease, endometriosis and evidence for microorganisms associated with PID among a cohort of clinically asymptomatic women undergoing laparoscopy or laparoscopy/fallopscopy for the investigation of infertility. The objective was to determine the prevalence of, and possible relationship between, asymptomatic infection and pathology of the fallopian tubes consequent to endometriosis and PID. This study reports on pathological findings at laparoscopy or laparoscopy/fallopscopy, and outcomes of micro-biological screening for microorganisms including *C. trachomatis*, *M. pneumoniae*, *M. hominis*, ureaplasma species, *N. gonorrhoeae* and *N. meningitidis*, and serological testing for *C. trachomatis*, *C. pneumoniae* and *M. pneumoniae*.

MATERIALS AND METHODS

Participants

This study involved 44 women presenting to a hospital IVF service for laparoscopic and fallopo-

scopic investigation of infertility. These women had a history of infertility of at least 2 years' duration, were clinically asymptomatic for PID, and either had a history of signs and symptoms suggestive of endometriosis, or a previous diagnosis of tubal disease as confirmed by previous laparoscopy, or longstanding unexplained infertility. All subjects had had other forms of infertility investigated (male factor, ovulation defects) before undergoing laparoscopy or laparoscopy/fallopscopy. Informed consent was obtained from all participants and the study was approved by the human ethics committees of the Queensland University of Technology and the Wesley Hospital, Brisbane, Queensland.

Clinical samples

Three separate clinical samples were collected from each of the participants: endocervical swabs for culture and PCR analysis; fallopian tube washings for culture, PCR analysis and serology; and 10 ml peripheral whole blood for serological assay. The endocervical swabs were collected during a speculum examination before the laparoscopy/fallopscopy procedure. The cervix was then prepared for fallopscopy and swabbed with povidone iodine. As a matter of protocol, all women were treated with antibiotics before the insertion of instrumentation through the cervix, in order to reduce the possibility of pelvic infection due to contamination carried from the vagina. Cefotaxime sodium, 2 g intravenously, was administered at the commencement of the procedure.

During fallopscopy an endoscope (Imagyn Medical, Switzerland) was inserted through the cervix and uterus into the fallopian tubes. The tubes were visualised along their length by means of a linear everting catheter and fallopscope. Saline was passed through the fallopian tube and any tissue debris or adhesions within the tubal lumen were cleared if possible during this procedure. The resultant cellular debris and saline were collected from the pouch of Douglas by aseptic aspiration via laparoscopy and aliquotted for testing. In 5 cases severe tubal pathology precluded fallopscopic examination, although saline was forced through under pressure. Thus

fallopian tube washings were collected in these 5 cases although falloposcopic observation was not possible.

Classification of endometriosis

The classification of endometriosis employed in this study was a modification of the American system, developed specifically with reference to fertility outcomes. Laparoscopic observations of endometriosis were classified according to the extent and distribution of endometriosis and the presence of adhesions. The presence of some peritoneal endometriosis deposits, with no endometriosis on tubes or ovaries and no pelvic adhesions, was classed as mild. The presence of peritoneal endometriosis deposits and/or pelvic adhesions, with superficial endometriosis deposits on the ovarian capsule but no periovarian or peritubal adhesions, was classed as moderate. Endometrioma formation in the ovaries, extensive peritoneal endometriosis, extensive pelvic adhesions, and deposits of endometriosis on tubal serosa with or without peritubal/periovarian adhesions constituted severe endometriosis.

Classification of intraluminal disease

Falloposcopy findings observed inside the fallopian tubes were classified as follows. Minimal loss of intraluminal folds with no adhesions or obstruction in either fallopian tube was classed as mild. Complete obstruction of one or incomplete obstruction of either one or both tubes, with moderate adhesions and moderate loss of tubal mucosal folds, was classed as moderate. Intraluminal tubal pathology (found at falloposcopy to be the primary cause of infertility) with permanent bilateral obstruction or extensive loss of normal intraluminal tubal architecture (presence of intratubal adhesions and loss of tubal mucosal folds) constituted severe disease.

Detection of *C. trachomatis* and neisseria species.

Endocervical swabs were tested by Amplicor CT/NG PCR (Roche Diagnostics, Branchburg, NJ, USA) for both *C. trachomatis* and *N.*

gonorrhoeae (according to the manufacturer's instructions), and cultured for *N. meningitidis* using chocolate agar incubated overnight at 35°C. Samples of fallopian tube washings obtained from falloposcopy and/or laparoscopy were also tested for *C. trachomatis* by the Roche Amplicor CT/NG PCR and cultured for both *N. gonorrhoeae* and *N. meningitidis* using Thayer Martin/chocolate agar. The Roche Amplicor CT/NG PCR method incorporates an internal control to monitor for the presence of PCR inhibitors.

Antibody screening

Peripheral blood serum and fallopian tube washings collected from participants were tested for anti-*C. trachomatis* IgG and IgA antibodies and anti-*C. pneumoniae* IgG antibodies. *C. trachomatis* antibodies were detected using the Labsystems *Chlamydia trachomatis* IgG EIA and IgA EIA (Helsinki, Finland). *C. pneumoniae* IgG antibodies were detected by immunofluorescence using the FOCUS Chlamydia MIF (Los Angeles, USA). An agglutination assay (Fujirebio Serodia-MYCOII, Japan) was selected for determination of *M. pneumoniae* antibodies using washings only.

Serum samples were tested and interpreted for *C. trachomatis* and *C. pneumoniae* antibodies according to the manufacturer's instructions. As no criteria were supplied for samples other than blood, the following adaptations were made for testing the fallopian fluids. Samples of *C. trachomatis* were initially tested undiluted; if positive, they were retested at dilutions of 1:2 and 1:10 prepared using sample diluent. *C. pneumoniae* samples were initially tested at 1:16 dilution; and *M. pneumoniae* samples were initially tested at 1:4 dilution.

Ureaplasma/ mycoplasma detection

Ureaplasma culture

Endocervical swabs (39 samples tested) and fallopian tube washings (40 samples tested) were inoculated directly into 10B broth¹⁶ and then held at -80°C until testing. In the laboratory,

four serial 10-fold dilutions were made of the liquid broth, which was then incubated at 37°C until ureaplasma growth was detected by an alkaline shift or for 3 weeks.

Ureaplasma/mycoplasma PCR detection

All clinical samples were prepared for extraction of DNA by the method of Blanchard *et al*¹⁷. All endocervical and fallopian tube clinical samples were tested by PCR assay for the presence of ureaplasmas. *M. hominis* and all endocervical ureaplasma isolates identified as *U. parvum* were further subtyped using four separate single-step *mba* PCR assays by previously described methods¹⁸. For *M. pneumoniae* testing, primers specific for the species were designed using the P1 and ORF453 genes of *M. pneumoniae* and an annealing temperature of 52°C. Primers were designed to avoid regions of high homology with *Mycoplasma genitalium* and *Ureaplasma urealyticum* sequences. ORF 453 encodes a possible protoporphyrinogen oxidase, and primers ORF-F (5' TCAACTACCCT-TACCCTATTACC 3') and ORF-R (5' GCACTCCAGTATCACTTGTT-TAAC 3') generate an amplicon of 505 bp. Primers to the P1 major adhesin of *M. pneumoniae* were designed in the constant region 1 to avoid repetitive elements¹⁹ and variable regions²⁰. PCR amplification using primers P1-F (5' CTTACTGT-TCTCAGGCATGG 3') and P1-R (5' TTGACAAGACCGTCCA-ATCC 3') produced a fragment of 383 bp. *M. pneumoniae* DNA kindly supplied by G. Browning, University of Melbourne, was used as a control. The PCR reaction mixture and thermal cycling conditions were those previously described¹⁸.

Other bacteriological culture

An endocervical swab was also cultured on horse blood and Sabouraud's dextrose agar and incubated overnight at 35°C.

Statistical analysis

Associations of chlamydia infection between women with nil/mild and moderate/severe endometriosis and tubal disease were examined by

two-way tables using chi-squared and Fisher's exact tests.

RESULTS

Laparoscopy and fallopscopy results

Of the 44 women enlisted in this study, 5 underwent a laparoscopy procedure but did not proceed to fallopscopy; however, in these cases fallopian tube washings were obtained as a result of saline being washed through. All of these 5 women were noted to have peritubal adhesions on external examination by laparoscopy, and their tubal disease was consequently classified as moderate to severe. On laparoscopy, 40 (90.9%) of the 44 women were found to have endometriosis which was classified as mild in 16 (36.4%) cases, as moderate in 15 (34.1%) cases, and as severe in 9 (20.5%) cases. The presence of fallopian tubal disease as identified by fallopscopy and/or external laparoscopy was identified as moderate to severe in 38/44 (86.4%) participants, and nil or mild in the remaining 6 (13.6%) participants. Of the other 4 women who recorded no diagnosis of endometriosis, all recorded severe tubal obstructions on fallopscopy; 7 women with mild endometriosis also showed severe, bilateral obstructions within the fallopian tubes.

Endocervical culture/PCR results

Ureaplasmas were detected by culture and PCR in 11/39 (28.2%) of the endocervical samples tested and were identified as *U. parvum*. These *U. parvum* isolates were subsequently subtyped; 7/12 (58%) were serovar 3 isolates, 1/12 (8.3%) were serovar 1, and 4/12 (33%) were serovar 6. Mixed serovars 1 and 3 were detected in 1 of the endocervical samples. *M. hominis*, *M. pneumoniae*, *C. trachomatis*, *N. gonorrhoeae* and *N. meningitidis* were not detected in any of the endocervical samples by PCR and/or culture. Culture of endocervical samples further identified mild growth of bacteroides species in 1/44 (2.3%) women, mild growth of *Candida albicans* in 1/44 women (2.3%), and moderate growth of group B streptococcus in 5/44 (11.4%) women, 1 of whom was coinfecting with *Gardnerella vaginalis*.

Endocervical cultures for the remaining women grew the bacterial flora that could normally be expected after vaginal contamination.

Fallopian tube culture/PCR results

C. trachomatis was detected in the fallopian tubes of 1 woman (Table 1). Serology for both fallopian washings and serum for this woman was found to be negative for *C. trachomatis*, and no other fallopian tube sample was found to be positive for *C. trachomatis*. Ureaplasmas, *M. hominis*, *M. pneumoniae*, *N. gonorrhoeae* and *N. meningitidis* were not detected in any of the fallopian tube samples.

Antibody testing

Both serum and fallopian tube fluids were available for serological testing for 38 of the 44 women; for 3 women only serum was tested and for 3 only fallopian tube fluid was tested.

Peripheral blood serology

Of the 41 serum samples, 4 (9.8%) were positive for *C. trachomatis* IgG, and 1 (2.4%) for *C. trachomatis* IgA (Table 1). Positive results for both IgG and IgA anti-*C. trachomatis* antibodies were found in 1 sample, and 1 demonstrated a positive IgG and an equivocal IgA anti-*C. trachomatis* antibody level; 25/36 samples (69.4%) were positive for *C. pneumoniae* IgG.

Fallopian tube serology

Of the 41 fallopian tube washings, 5 (12.2%) were positive for the presence of *C. trachomatis* IgG, 1 (2.4%) for *C. trachomatis* IgA (Table 1), 5 (12.2%) for *C. pneumoniae* IgG, and 1 (2.4%) for *M. pneumoniae* serology. In 1 case both IgG and IgA anti-*C. trachomatis* antibodies were found in both the fallopian tube and serum.

Association of laparoscopic and falloposcopic findings with serology/microbiological results

Evidence (either in peripheral blood or fallopian tube washings) of *C. trachomatis* infection was found in 7 women (Table 1). Nil or mild endometriosis was diagnosed in 5 (71%) of these, and 4 of the 5 were also shown to have moderate to severe intraluminal disease according to falloposcopic observation. Participant 32 (Table 1) showed no evidence of endometriosis but had severe tubal disease with extensive bilateral damage. Only participants 14 and 31 (Table 1) had endometriosis classified as moderate and severe, respectively, and both these women had severe tubal disease showing extensive adhesions and scarring. Participant 14 was also the 1 woman with a *C. trachomatis* infection in the fallopian tube detectable by PCR, although serology was negative. Therefore, in total, 6 (86%) of the 7 women with evidence of chlamydial infection showed moderate to severe intraluminal disease

Table 1 Combined results of serum and fallopian tube *C. trachomatis* pathology

Case	Serum IgG	Serum IgA	Fallopian IgG	Fallopian IgA	Endometriosis	Intraluminal disease
Patient 12	+	-	+	-	Mild	Moderate
Patient 14, PCR + ve	-	-	-	-	Moderate	Severe
Patient 17	+	Equivocal	-	-	Mild	Severe
Patient 29	+	-	+	-	Mild	Nil
Patient 31	-	-	+	-	Severe	Severe
Patient 32	+	+	+	+	Nil	Severe
Patient 36	-	-	+	-	Mild	Severe
Total positives	4	1	5	1		
Total patients	41	41	41	41		
Percentage + ve	9.8%	2.4%	12.2%	2.4%		

but only 2 (28.6%) of these 7 women had moderate to severe endometriosis.

In 1 case with positive mycoplasma serology (fallopian tube fluid), mild endometriosis was defined, with severe tubal disease and a history of ruptured appendicitis.

Of the remaining 37 women who showed no microbiological evidence of chlamydial infection, 11 (29.7%) had a diagnosis of mild endometriosis, 13 (35.1%) of moderate endometriosis, and 9 (24.3%) of severe endometriosis, whereas 4 (10.8%) had no endometriosis. According to falposcopic observations, 5 (13.5%) of women in this same group had no or mild tubal disease and 32 (86.5%) had moderate to severe tubal disease.

The laparoscopy and falposcopy results for those women with *C. trachomatis* positivity, and those with none, were grouped into two general categories of endometriosis and tubal disease (nil/mild and moderate/severe) and are described in Table 2.

Among the women with evidence of chlamydial infection, 71% had a diagnosis of nil/mild endometriosis diagnosis compared with 40.5% of women with no evidence of chlamydia ($p > 0.5$). Among the women with evidence of chlamydia and classifications of nil/mild endometriosis, 56.7% showed moderate/severe tubal damage compared with 32.4% of women without positive chlamydia serology and with nil/mild endometriosis ($p > 0.5$).

The 9 women with endocervical ureaplasma infection were evenly distributed across both

groups of endometriosis: 44% had nil/mild endometriosis and 56% had moderate/severe endometriosis.

A review of the clinical histories of the participants showed that of the 7 women with evidence of *C. trachomatis* infection, 1 gave a history of miscarriage, 1 gave a history of ectopic pregnancy, and 2 had had PID previously. Another 2 participants in this group displayed significant tubal disease indicative of previous infection. In comparison, the clinical histories of the other 37 women included (in addition to endometriosis) 5 cases of polycystic ovarian disease, 4 of irregularities with ovulation, 1 of possible PID at a very early age (no tubal disease was detected), 1 of hydrosalpinx, 1 of suspected infection from a previous termination of pregnancy and 2 of appendicitis.

When serum samples were assayed for *C. pneumoniae*, equivalent proportions of patients with either nil/mild or moderate/severe endometriosis were found to have positive serology for *C. pneumoniae* (44% and 56%, respectively).

DISCUSSION

C. trachomatis is now recognised as one of the most common sexually transmissible bacterial pathogens throughout the world. *C. trachomatis* has previously been found to be the primary cause of salpingitis and pelvic inflammatory disease (30 to 60% of cases)^{7-10,21}, often with consequent ectopic pregnancy²² and tubal infertility^{23,24}. The presence of *C. trachomatis* DNA at sites of inflammation and tissue damage within reproductive tissue in both animal models²⁵ and humans with PID and tubal infertility²⁶⁻²⁹ suggests that chlamydia persist within the fallopian tube in a non-replicative chronic state and may be directly involved in the stimulation of the immune-mediated tissue destruction.

However, despite strong evidence of *C. trachomatis* involvement in tubal infertility, isolation of the organism by culture is rare²⁸. Falposcopy as performed for this cohort of women remains one of the few options for obtaining fallopian tissue for analyses. However, in our study tissue biopsies were not taken. In view of the intracellular reproductive cycle of *C.*

Table 2 *C. trachomatis* pathology in different categories of endometriosis and intraluminal disease: numbers (%) of cases

Tubal disease	Nil/mild endometriosis	Moderate/ severe endometriosis
Positive for <i>C. trachomatis</i>		
-nil/mild	1 (14.3%)	—
-moderate/severe	4 (56.7%)	2 (29.0%)
-total (n = 7)	5 (71.0%)	2 (29.0%)
Negative for <i>C. trachomatis</i>		
-nil/mild	3 (8.1%)	2 (5.5%)
-moderate/severe	12 (32.4%)	20 (54.0%)
-total (n = 37)	15 (40.5%)	22 (59.5%)

trachomatis, the collection of luminal tissue for testing is ideal, but the process of clearing out debris by means of the passage of saline through the fallopian tube was considered sufficient in our study to provide suitable material for PCR testing. Nonetheless, one deficiency in this approach was our failure to collect pre-existing *cul-de-sac* fluid before aspiration of the tubal effluent.

This study reports the microbiological screening of cervical, serum and fallopian tube samples collected from women undergoing laparoscopic and/or falposcopic investigation for infertility. A total of 7 women (15.9%) within our cohort of 44 women showed evidence of previous *C. trachomatis* infection, either in the form of serum IgG ($n=4$) and/or IgA antibodies ($n=1$), fallopian tube IgG ($n=5$) and/or IgA ($n=1$) antibodies, or chlamydial DNA detected by PCR in the fallopian tube washings ($n=1$). This prevalence, recorded in a population of women accessing an infertility service, is as expected higher than both the general and antenatal prevalence rates. The prevalence of 3 to 5% in the general community was calculated from urine-based PCR among, for example, adult clients attending the Brisbane Sexual Health Clinic on any given month (unpublished data). The antenatal prevalence of 3.7% was previously reported in antenatal patients attending a public hospital clinic³⁰.

The prevalence (15.9%) found in this study is comparable with that reported by other studies. In a previous study of 1303 subfertile couples, asymptomatic for genital tract infection, elevated titres of IgG anti-chlamydial antibodies were found in 20.8% of all women¹¹. Similarly Vogelzang *et al.*³¹, in a study of infertile women referred to an IVF provider for diagnostic laparoscopy, found that 7/33 (21.2%) women with tubal disorders (adhesions, distal occlusions and strictures) demonstrated antibodies to *C. trachomatis* compared with 0/20 of women without tubal damage. Further studies have also strongly supported the link between serological evidence of previous *C. trachomatis* infections and tubal infertility and ectopic pregnancies^{12–15}.

The detection of antibodies to *C. trachomatis* within fallopian tube washings collected does not

necessarily reflect actual antibody production in the tubes but could also be the result of serum transudate or contamination by blood washed through with the dislodged cellular debris and fragments of endometrium.

In this current study we also compared the prevalence and severity of endometriosis in women positive and negative for *C. trachomatis*. Although there was no statistical significance, there appeared to be a trend in which a higher proportion of those infertility clinic clients returning a positive *C. trachomatis* diagnosis recorded nil/mild categories of endometriosis (5/7: 71%) compared with those women without any evidence for *C. trachomatis* (15/37: 40.5%). Also, there was a trend in which a lower proportion of positive *C. trachomatis* diagnoses recorded moderate/severe categories of endometriosis (2/7: 29%) compared with the negative group (22/37: 59.5%). Again this was not significant in view of the small numbers. These results suggest a negative relationship between *C. trachomatis* and endometriosis. Therefore, in this current study, endometriosis appears not to be associated with a previous history of *C. trachomatis* infection. A previous study had similarly found that only 1/15 patients with endometriosis had detectable antibodies to *C. trachomatis*¹⁴.

The majority of participants in this present study were found during falposcopy to have moderate/severe intraluminal disease (38/44: 86.4%). Moderate/severe tubal damage was observed in women with both nil/mild and moderate/severe endometriosis (87.1% and 80.4%, respectively). Therefore the severity of the tubal damage did not relate to the endometriosis classification. 16/44 (36%) of the women in our study presented with moderate to severe tubal damage in the absence of moderate to severe endometriosis. Although 25% (4/16) of these women showed evidence of *C. trachomatis* infection, the aetiology of tubal damage for the remaining 12 women remained undefined.

When both endometriosis and tubal disease were investigated together, 56.7% of participants with positive *C. trachomatis* findings had moderate/severe tubal disease and nil/mild endometriosis, whereas only 32.4% of participants without evidence of *C. trachomatis* infection had

similar categories of intraluminal disease and endometriosis. Therefore the findings of tubal damage in those women with evidence of *C. trachomatis* may be due to this previous infection, in view of the fact that endometriosis was predominantly mild or non-existent for this group.

A more speculative explanation for the higher prevalence of *C. trachomatis* among this small sample of women with nil/mild endometriosis may be a protective effect. Could *C. trachomatis* infection limit the extent and severity of endometriosis or, conversely, does a pre-existing endometriosis condition limit *C. trachomatis* infection? It may be possible that tubal occlusion resulting from *C. trachomatis* infection may create a physical barrier against endometrial tissue passing through the fallopian tube or, alternatively, immune responses generated during endometriosis such as elevated cytokine expression^{2,32,33}, increased neutrophil activity^{34–36} and increased phagocytic activity^{37,38} may clear chlamydial colonisation before the development of florid infection.

The prevalence of *C. pneumoniae* antibodies in sera was evenly distributed across both classifications of endometriosis (Table 3), and there did not appear to be an association with *C. trachomatis* serology. Freidank *et al.*³⁹ showed a higher anti-hsp60 antibody rate in patients with complete tubal occlusion (76%) than in infertility patients with normal fallopian tubes (19%), and this was associated with the presence of antibodies not only to *C. trachomatis* but also to *C. pneumoniae*, suggesting a synergistic effect due to cross-reactivity. However, Osseur and Persson⁴⁰ had earlier argued against the possibility of cross-reactivity due to shared antigens by demonstrating that antibodies to *C. pneumoniae* did not influence the frequency or titre of

antibodies to *C. trachomatis* in patients recovering from acute salpingitis. *C. trachomatis* antibodies, however, did reflect the severity of inflammatory involvement. More recently⁴¹, patients with tubal factor infertility were shown to have significantly higher frequencies and titres for all antibodies (*C. trachomatis* hsp60, hsp10, common chlamydial lipopolysaccharide) except to *C. pneumoniae*. No interaction between *C. trachomatis* and *C. pneumoniae* suggestive of a synergistic effect was found. The results of our study would suggest a similar trend, although the numbers of patients were too small for statistical significance.

In this study we also tested endocervical swabs and fallopian tube washings for the presence of the ureaplasmas (*U. parvum*, *U. urealyticum*), *M. hominis* and *M. pneumoniae*. Whereas *M. hominis* and *M. pneumoniae* were not detected in either endocervical or fallopian tube samples, 28% of the women tested were found to have *U. parvum* in the endocervix. However, no ureaplasmas were detected in the fallopian tube washings by either culture or PCR. Testing of the fallopian tube specimens using a more sensitive nested *mba* PCR assay (results not shown) also failed to detect ureaplasmas within these specimens. These results confirm that the fallopian tube specimens had not been contaminated during the transcervical fallopscopy procedure.

Previous studies demonstrated a very low isolation rate of the ureaplasmas (0 to 3%) and *M. hominis* (0 to 4%) from the fallopian tubes^{7,9}. This is despite higher ureaplasma/*M. hominis* colonisation rates in the endocervix (48 to 52%) and the endometrium (6 to 30%). However, these studies highlighted the increased prevalence of *C. trachomatis*: 30% of women were infected with *C. trachomatis* in the endometrium and 17 to 22% of women demonstrated infection within fallopian tubes^{7,9}. *M. pneumoniae* was not detected in either endocervical or fallopian tube samples in this current study, but this microorganism has been associated with orogenital contact and has been isolated from cervical and male urethral swabs⁴². A rare case of tubo-ovarian abscess has also been reported after pneumonia, indicating the possibility of systemic spread⁴³.

Table 3 Correlation of *C. pneumoniae* serology with endometriosis rating: numbers (%) of cases

Endometriosis rating	Positive <i>C. pneumoniae</i> serology (n = 25)	Negative <i>C. pneumoniae</i> serology (n = 11)
Nil/mild	11 (44%)	6 (55%)
Moderate/severe	14 (56%)	5 (45%)

Our study also shows that there is no association between the prevalence of endometriosis and *U. parvum*, given that 44.4% of women with endocervical ureaplasmas had nil/mild endometriosis compared with 55.6% of ureaplasma-positive women with moderate/severe endometriosis.

N. meningitidis, another respiratory pathogen and coloniser, has also been isolated from the female genitourinary tract in both symptomatic and asymptomatic states, and is generally associated with orogenital contact⁴³. Symptomatic infections range from urethritis to purulent cervicitis and salpingitis. In our population sample of 44 women, no *N. meningitidis* was isolated from either cervix or fallopian tube by culture. Whereas the prevalence of anogenital infection in the UK has remained very low at 0.13%, other studies in the USA have isolated *N. meningitidis* in 20% of cases urogenital detection of pathogenic *Neisseria species*⁴³.

Other organisms identified in the endocervix of these women were considered consistent with lower genital tract flora, as no participants in the study had symptoms of PID or vaginitis.

The evidence for *C. trachomatis* infection identified in 15.9% of women involved in this study was comparable with previous studies and was certainly higher than the prevalence (3.7%) within a population of fertile Australian women³⁰. The absence of *C. trachomatis* indicators in women with moderate to severe endometriosis,

and the comparable rates of women with and without evidence for ureaplasma across all groupings of endometriosis, supports a non-microbial cause for endometriosis. Further, this study may suggest a protective effect by which previous chlamydial-induced tubal damage may limit the spread of endometriosis, or immune responses associated with endometriosis may prevent chlamydia infection. Our study also suggested that there was no synergistic link between *C. pneumoniae* and *C. trachomatis* infection with respect to inducing tubal damage. Finally, a third of the women in our group who presented with moderate to severe intraluminal disease had no significant evidence of endometriosis. Whereas 25% of this group showed some evidence for previous *C. trachomatis* infection, there remained a majority with no apparent microbiological cause for their tubal disease.

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